Reversal of Propranolol Blockade of Adrenergic Receptors and Related Toxicity with Drugs that Increase Cyclic AMP (44422)

V. E. WHITEHURST,*[1] J. A. VICK,† F. R. ALLEVA,‡ J. ZHANG,† X. JOSEPH,§ AND T. BALAZS‡

Division of Pulmonary Drug Products, Food and Drug Administration, Rockville, Maryland 20857; Division of Applied Pharmacology and Research,† Food and Drug Administration, Beltsville, Maryland 20705; Division of Drug Biology,‡ Food and Drug Administration, Washington, D.C. 20204; and Division of Cardio-Renal Drug Products,§ Food and Drug Administration, Rockville, Maryland 20857*

Abstract. **An overdose of propranolol, a widely used nonselective β-adrenergic receptor blocking agent, can result in hypotension and bradycardia leading to irreversible shock and death. In addition, the blockade of adrenergic receptors can lead to alterations in neurotransmitter receptors resulting in the interruption of the activity of other second messengers and the ultimate cellular responses. In the present experiment, three agents, aminophylline, amrinone, and forskolin were tested in an attempt to reverse the potential lethal effects of a propranolol overdose in dogs. Twenty-two anesthetized beagle dogs were given a 10-min infusion of propranolol at a dose of 1 mg/kg/min. Six of the dogs, treated only with intravenous saline, served as controls. Within 15–30 min all six control dogs exhibited profound hypotension and severe bradycardia that led to cardiogenic shock and death. Seven dogs were treated with intravenous aminophylline 20 mg/kg 5 min after the end of the propranolol infusion. Within 10–15 min heart rate and systemic arterial blood pressure returned to near control levels, and all seven dogs survived. Intravenous amrinone (2–3 mg/kg) given to five dogs, and forskolin (1–2 mg/kg) given to four dogs, also increased heart rate and systemic arterial blood pressure but the recovery of these parameters was appreciably slower than that seen with aminophylline. All of these animals also survived with no apparent adverse effects. Histopathologic evaluation of the hearts of the dogs treated with aminophylline showed less damage (vacuolization, inflammation, hemorrhage) than the hearts from animals given propranolol alone. Results of this study showed that these three drugs, all of which increase cyclic AMP, are capable of reversing the otherwise lethal effects of a propranolol overdose in dogs.**

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Propranolol, a β-adrenergic receptor antagonist and Captopril, an angiotensin-converting enzyme inhibitor, have been shown to be beneficial in the treatment of heart failure (1). These drugs improve the hemodynamic and clinical responses in patients with idiopathic dilated cardiomyopathy by protecting the myocardium from the cardiotoxic effects of increased catecholamines and by up-regulating adrenergic receptors (2). This approach to treating human heart failure is based on the hypothesis that continuous adrenergic stimulation is detrimental and perhaps even counterproductive in chronic forms of cardiac decomposition (3). This hypothesis is supported by studies in diabetic rats and mice (4, 5).

During normal physiologic and stressful conditions, β-adrenoreceptors play a very important role in the electrophysiologic properties of the myocardium as well as in the physiology of airway smooth muscle in the pulmonary system by their ability to activate the production of second messengers, cyclic AMP (cAMP), diacylglycerol and phosphoinositols, *via* G-proteins and special enzymes (i.e., adenylate cyclase or phospholipase). The blockade of the β-ad-

[1] To whom requests for reprints should be addressed at FDA (HFD-570), Parklawn Building, 5600 Fishers Lane, Rockville, MD 20857. E-mail: Whitehurst@CDER.FDA.GOV

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renergic receptors prevents communication between the receptor and the enzyme system eliminating or greatly reducing the production of second messengers and eliminating and/or changing the cellular response. Obstruction of the β-adrenergic receptors can also lead to hypotension, excess negative inotropism, and bradycardia leading to shock and sudden death in rats and mice (6). Additionally, blockade of the β-adrenergic receptors in the smooth muscle of the lung can lead to increased airway hyperreactivity, increased aerosol antigen-induced bronchoconstriction and pulmonary eosinophilia as measured by bronchoalveolar lavage (7).

Chronic exposure of the adrenergic receptors can also lead to an abatement in the number of adrenergic receptors in lymphocyte subsets after exercise (8). It has been shown that in the failing human heart, β-adrenergic receptors undergo agonist-mediated downregulation. The downregulation of the receptor is closely correlated with the downregulation of its mRNA, an effect controlled in part by the stability of the mRNA (9). The decrease in the quantity of receptors is due to internalization (desensitization) or, in the case of the myocyte, uncoupling of the receptors (6). The loss of the availability of the receptor is an attempt to diminish neurotransmitter stimulation that can lead to increased energy demands on the cell.

The major objective of this paper therefore is to compare the ability of three different drugs to enlist an inotropic response and prevent death in animals when the adrenergic receptors are blocked by the nonselective beta-blocker, propranolol. The drugs used in this study are aminophylline, a nonspecific phosphodiesterase inhibitor, amrinone, a selective phosphodiesterase inhibitor, and forskolin, a novel activator of the catalytic subunit of adenylate cyclase.

Materials and Methods

Twenty-two adult beagle dogs 2–7 years of age, bred at the FDA Beltsville Research Facility, and weighing 8.5–12.5 kg were used in this study. Dogs were anesthetized with intravenous sodium pentobarbital, 30 mg/kg, and instrumented to record arterial and venous blood pressure, lead II electrocardiogram (EKG), and heart rate. Respiratory function was monitored using a flow-through Pneomotach connected to a standard endotracheal tube. Permanent recordings were made on a Model 350 Sanborn Polygraph.

Following a 30-min period of equilibration, all dogs were given an intravenous infusion of propranolol HCl at a dose rate of 1 mg/kg/min for 10 min. The infusion was given directly into the femoral vein. The six control dogs were given only the infusion of propranolol. Aminophylline, 20 mg/kg, amrinone 2–3 mg/kg, or forskolin 1–2 mg/kg were given intravenously to seven, five, and four dogs, respectively, at approximately 5–10 min after the propranolol infusion.

Following the study, all surviving animals were placed in recovery cages and observed for 72 hr at which time the surviving dogs were euthanized with a high concentration of sodium pentobarbital. Hearts were removed from six propranolol (control) dogs, four aminophylline, four amrinone, and two forskolin-treated dogs and placed in formalin for subsequent histopathologic examination.

All drugs used in this study were obtained from Sigma Chemical Company (St. Louis, MO) and were administered in sterile saline.

The animals used in this study were approved by the institution's Animal Care and Use Committee.

Results

The six control dogs given only the infusion of propranolol showed progressive cardiovascular deterioration characterized by bradycardia, EKG changes, respiratory distress, and hypotension (Fig. 1). All of the control dogs died within 15–30 min following the infusion of propranolol. In contrast, the seven dogs treated with aminophylline made a remarkable recovery from the severe cardiovascular/respiratory distress occurring within 1–5 min following drug treatment. Both heart rate and blood pressure increased immediately, and respiration improved, both in rate and volume exchange (Fig. 2). At 24 and at 72 hr, all aminophylline-treated dogs appeared normal.

The effects of treatment with amrinone (Fig. 3) or forskolin (Fig. 4) were similar to those described for aminophylline except that the recovery time was considerably slower (i.e., heart rate and blood pressure remained low for approximately 10 min and was followed by a slow progressive return of monitored parameters to control or near control values in about 25 min). As with aminophylline, all five amrinone- and four forskolin-treated dogs survived the 72 hour observation period.

Microscopic analyses of the hearts of the dogs in the propranolol-treated group revealed vacuolization, inflam-

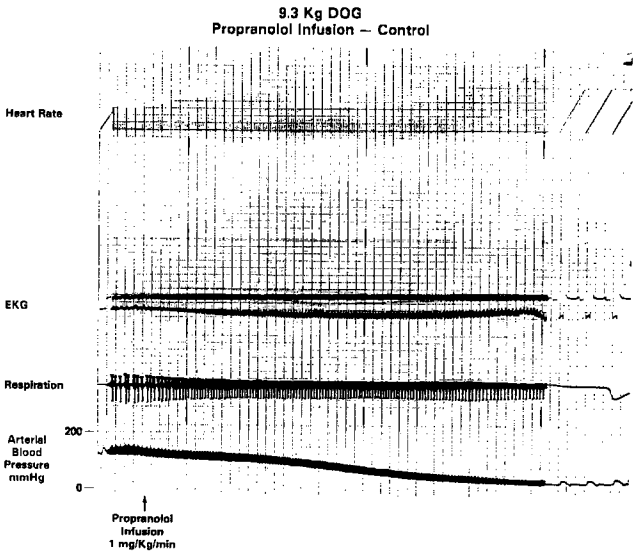

Figure 1. Effect of propranolol infusion on heart rate, EKG, respiration, and arterial blood pressure in the anesthetized dog preparation.

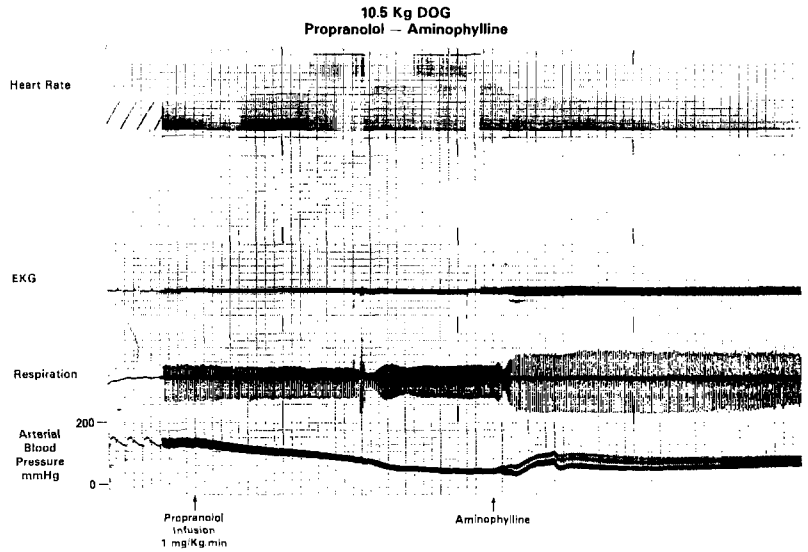

Figure 2. Effect of propranolol infusion and treatment with aminophylline on heart rate, EKG, respiration, and arterial blood pressure in the anesthetized dog preparation.

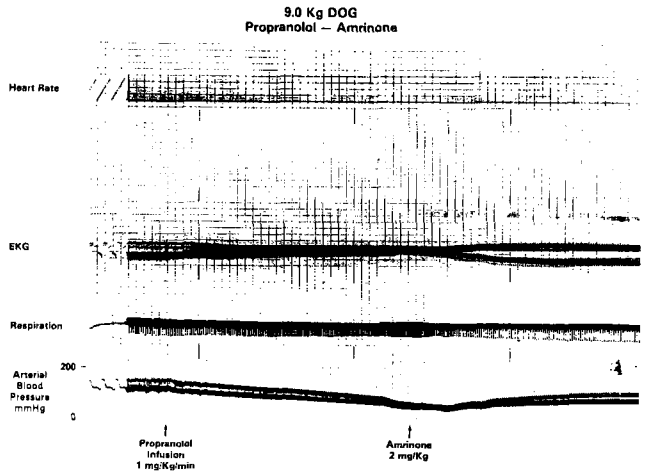

Figure 3. Effect of propranolol infusion and treatment with amrinone on heart rate, EKG, respiration, and arterial blood pressure in the anesthetized dog preparation.

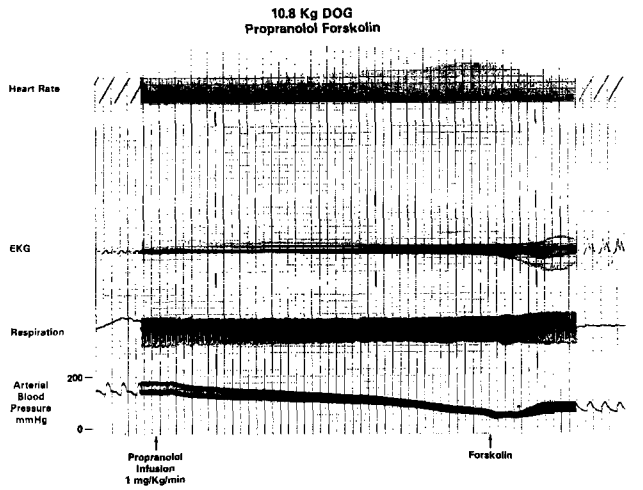

Figure 4. Effect of propranolol infusion and treatment with forskolin on heart rate, EKG, respiration, and arterial blood pressure in the anesthetized dog preparation.

mation, and hemorrhage (Fig. 5). Numerous inflammatory cells, including macrophages and lymphocytes were distributed in the epicardium and endocardium that was identified with Masson Trichrome stain. Pathology scores in this group of animals were 2/6 (+1), 3/6 (+2), and 1/6 (+3).

Tissues from dogs treated with aminophylline, amrinone, and forskolin showed far less damage than the hearts from untreated controls. Some mild inflammation and hemorrhage was seen in these animals, however, the combined pathology scores were low; 1/10 (+0), 9/10 (+1), 0/10 (+2), and 0/10 (+3). It is interesting to compare these results with those of the propranolol treated dogs which had path scores of +2, or +3 in 4 of 6 animals.

Discussion

Results of this study indicate that three pharmacologically different drugs, namely, aminophylline, amrinone, and forskolin, are all capable of reversing the otherwise lethal effects of propranolol overdose in the dog.

Our results indicate that aminophylline is indeed superior to both amrinone and forskolin in rapidly overcoming the propranolol-induced depression of myocardial function. Prompt restoration of blood pressure and heart rate was observed in the aminophylline-treated dogs compared to a somewhat slower response seen with forskolin and amrinone. These findings are extraordinary in light of studies reported by Wolff *et al.* (10) in that ischemic perturbations and/or abnormal function of G protein (Gs) tend to decrease the isoproterenol-stimulated adenylyl cyclase activity, reduce β-myocardial density, and cause a marked decrease in agonist affinity. However, this investigation also suggests that it is possible that the signal amplication between the β-adrenergic receptors and the Gs may be more efficient during the first 30–60 min of ischemia.

Recent reports reveal that myocardial β-adrenoreceptor density may increase or decrease in response to a variety of pharmacologic and pathophysiologic stimuli (10, 11). Vatner *et al.* reported an increase in β-adrenoceptor density in

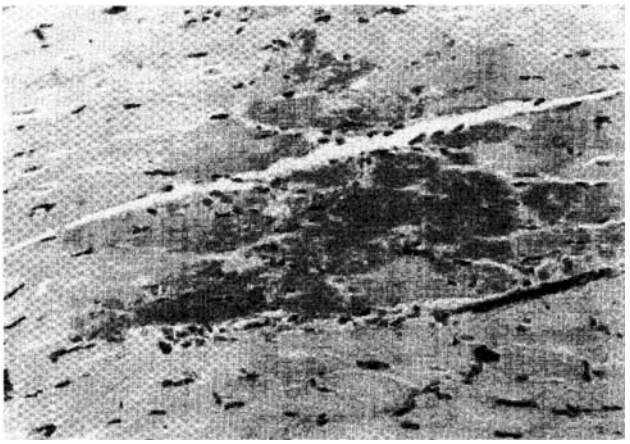

Figure 5. Effect of propranolol overdose on the histopathology of the anesthetized dog.

the intact canine model in which the non ischemic myocardium was kept *in situ* for an hour after death (12). Delehanty *et al.* also reported a significant decrease in myocardial β-adrenoceptor density in fast-paced dogs (13).

The altered response induced by propranolol may lead to a more effective interaction of the reduced number of β-adrenoceptors and a higher concentration of catecholamines. The adenylyl activity may likewise be potentiated by aminophylline.

The prompt restoration of cardiac functions observed in the dogs treated with aminophylline may be due to the ability of the drug to increase leakage of calcium from the sarcoplasmic reticulum (SR). Both *in vivo* and *in vitro* studies reveal that phosphodiesterase inhibitors do have the ability to cause passive leakage of calcium from the SR as well as increase the rate of activator calcium SR (14, 15).

Other investigations have also shown that in heart failure and in acute myocardial ischemia, alterations in the neurotransmitter system may extend beyond the receptor and involve both the stimulatory and the inhibitory families as well as the catalytic subunit of the adenylyl cyclase (11, 16). Our data also confirm that the use of forskolin and amrinone are effective in abating postreceptor catalytic subunit dysfunction. The mechanism by which these drugs initiate inotropic responses is unclear.

The findings with aminophylline suggest that this phosphodiesterase inhibitor could possibly be the drug of choice in treating emergency situations resulting from propranolol overdose. Additional studies are needed to confirm and expand these findings and to determine whether phosphodiesterase inhibitors could be used in the human.

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